DB Name	Query	Hit Count	Set Name
USPT,JPAB,EPAB,DWPI,TDBD	l16 and melanoma and bladder	22	L17
USPT,JPAB,EPAB,DWPI,TDBD	l15 and "antigen presenting cell$"	80	L16
USPT,JPAB,EPAB,DWPI,TDBD	"adoptive immunotherapy$" and cancer$ and virus$	221	L15
USPT,JPAB,EPAB,DWPI,TDBD	"adoptive immunotherapy$"	406	L14
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 vir$ adj10 prolifer$	3	L13
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USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 cancer$ adj10 prolifer$	6	L11
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 cancer$	144	L10
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 proliferation adj10 stimulat$	18	L9
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 proliferation adj10 therap$	6	L8
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj5 proliferation adj10 culture$	7	L7
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj5 proliferation	180	L6
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 proliferation	231	L5
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj10 proliferation adj10 "in vitro"	0	L4
USPT,JPAB,EPAB,DWPI,TDBD	CTL$ adj5 proliferation adj5 "in vitro"	0	L3
USPT,JPAB,EPAB,DWPI,TDBD	l1 and proliferation	1517	L2
USPT,JPAB,EPAB,DWPI,TDBD	"cytotoxic T lymphocyte$" or CTL$	8380	L1

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l16 and melanoma and bladder	22

Database: US Patents Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: 116 and melanoma and bladder — Clear

Search History

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☐ 1. Document ID: US 6034214 A

L8: Entry 1 of 6 File: USPT Mar 7, 2000

US-PAT-NO: 6034214
DOCUMENT-IDENTIFIER: US 6034214 A

TITLE: Isolated nonapeptides which bind to HLA molecules and provoke lysis by cytolytic T cells

DATE-ISSUED: March 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boon; Thierry	Brussels	N/A	N/A	BEX
van der Bruggen; Pierre	Brussels	N/A	N/A	BEX
De Plaen; Etienne	Brussels	N/A	N/A	BEX
Lurquin; Christophe	Brussels	N/A	N/A	BEX
Traversari; Catia	Milan	N/A	N/A	ITX

US-CL-CURRENT: 530/328; 424/185.1, 530/300, 530/385

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

☑ 2. Document ID: US 5831068 A

L8: Entry 2 of 6 File: USPT Nov 3, 1998

US-PAT-NO: 5831068
DOCUMENT-IDENTIFIER: US 5831068 A

TITLE: Method to increase the density of antigen on antigen presenting cells

DATE-ISSUED: November 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nair; Smita K.	Durham	NC	N/A	N/A
Gilboa; Eli	Durham	NC	N/A	N/A

US-CL-CURRENT: 536/24.5; 424/278.1, 435/325, 435/343.2, 435/375

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 5695994 A

L8: Entry 3 of 6 File: USPT Dec 9, 1997

US-PAT-NO: 5695994
DOCUMENT-IDENTIFIER: US 5695994 A

TITLE: Isolated cytolytic T cells specific for complexes of MAGE related peptides and HLA molecules

DATE-ISSUED: December 9, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boon-Falleur; Thierry	Brussels	N/A	N/A	BEX
van der Bruggen; Pierre	Brussels	N/A	N/A	BEX
De Plaen; Etienne	Brussels	N/A	N/A	BEX
Lurguin; Christophe	Brussels	N/A	N/A	BEX
Traversari; Catia	Milan	N/A	N/A	ITX
Gaugler; Beatrice	Brussels	N/A	N/A	BEX
Van den Eynde; Benoit	Brussels	N/A	N/A	BEX

US-CL-CURRENT: 435/325; 435/355, 435/372.3, 530/328

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 5462871 A

L8: Entry 4 of 6 File: USPT Oct 31, 1995

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L8: Entry 2 of 6 File: USPT Nov 3, 1998

DOCUMENT-IDENTIFIER: US 5831068 A
TITLE: Method to increase the density of antigen on antigen presenting cells

ABPL:
Disclosed is a method for presenting an antigen in the form of a peptide on the surface of a cell. The method involves inhibiting the activity of an MHC class I pathway-associated component (e.g., a TAP protein or a proteasome or its components) in a cell and contacting the cell with an antigenic peptide to produce a potent antigen presenting cell. The antigen presenting cells of the invention can be administered to a mammal in a method of treating or preventing cancer or infection with a pathogen (e.g., a bacterium or virus). If desired, the antigen presenting cells can be used to stimulate CTL proliferation in vitro, and the resulting effector cells can subsequently be administered to a mammal in a method of therapy.

US-PAT-NO: 5462871
DOCUMENT-IDENTIFIER: US 5462871 A

TITLE: Isolated nucleic acid molecules which encode MAGE derived nonapeptides

DATE-ISSUED: October 31, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boon-Falleur; Thierry	Brussels	N/A	N/A	BEX
van der Bruggen; Pierre	Brussels	N/A	N/A	BEX
De Plaen; Etienne	Brussels	N/A	N/A	BEX
Lurquin; Christophe	Brussels	N/A	N/A	BEX
Traversari; Catia	Milan	N/A	N/A	ITX
Gaugler; Beatrice	Brussels	N/A	N/A	BEX
Van den Eynde; Benoit	Brussels	N/A	N/A	BEX

US-CL-CURRENT: 435/354; 435/252.3, 435/365, 536/23.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 5405940 A

L8: Entry 5 of 6 File: USPT Apr 11, 1995

US-PAT-NO: 5405940
DOCUMENT-IDENTIFIER: US 5405940 A

TITLE: Isolated nonapeptides derived from MAGE genes and uses thereof

DATE-ISSUED: April 11, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boon; Thierry	Brussels	N/A	N/A	BEX
van der Bruggen; Pierre	Brussels	N/A	N/A	BEX
De Plaen; Etienne	Brussels	N/A	N/A	BEX
Lurquin; Christophe	Brussels	N/A	N/A	BEX
Traversari; Catia	Milan	N/A	N/A	ITX

US-CL-CURRENT: 530/328; 424/185.1, 530/300

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: WO 9707128 A1

L8: Entry 6 of 6 File: EPAB Feb 27, 1997

PUB-NO: WO009707128A1
DOCUMENT-IDENTIFIER: WO 9707128 A1
TITLE: A METHOD TO INCREASE THE DENSITY OF ANTIGEN ON ANTIGEN PRESENTING CELLS

PUBN-DATE: February 27, 1997

INVENTOR-INFORMATION:

NAME	COUNTRY
NAIR, SMITA K	N/A
GILBOA, ELI	N/A

INT-CL (IPC): C07H 21/04; A61K 31/00; A61K 48/00

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CTL$ adj10 proliferation adj10 therap$	6

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L11: Entry 1 of 6 File: USPT Feb 1, 2000

US-PAT-NO: 6019987
DOCUMENT-IDENTIFIER: US 6019987 A

TITLE: Isolated, MAGE-3 derived peptides which complex with HLA-A2 molecules and uses thereof

DATE-ISSUED: February 1, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Van Der Bruggen; Pierre	Brussels	N/A	N/A	BEX
Boon-Falleur; Thierry	Brussels	N/A	N/A	BEX
Traversari; Catia	Milan	N/A	N/A	ITX
Fleischauer; Katharina	Milan	N/A	N/A	ITX

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Ludwig Institute for Cancer Research	New York	NY	N/A	N/A	02

APPL-NO: 8/ 668560
DATE FILED: June 21, 1996

PARENT-CASE:
This application is a Divisional of Ser. No. 08/217,187 filed Mar. 24, 1994 now U.S. Pat. No. 5,554,506.

INT-CL: [6] A61K 39/00, C12N 15/85, C07K 16/00
US-CL-ISSUED: 424/277.1; 424/185.1, 424/193.1, 435/7.1, 435/372.3, 435/7.23, 350/388.8, 350/387.1, 514/15, 436/506, 530/388.1
US-CL-CURRENT: 424/277.1; 424/185.1, 424/193.1, 435/372.3, 435/7.1, 435/7.23, 436/506, 514/15, 530/387.1, 530/388.1, 530/388.8
FIELD-OF-SEARCH: 424/185.1, 424/193.1, 424/277.1, 350/387.1, 350/388.8, 514/15, 435/7.1, 435/7.23, 435/372.3, 530/388.1, 436/506

REF-CITED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5342774	August 1994	Boon et al.	N/A

OTHER PUBLICATIONS

Engelhard, V., Curr. Opin. Immunol. 6:13-23, "Structure of peptides associated with MHC Class I molecules", 1994.
Rammensee et al., Annu. Rev. Immunol. 11:213-244, Peptides naturally presented by MHC Class I molecules, 1993.
Rammensee et al., Immunogenetics 41:178-228, "MHC ligands and peptide motifs: first listing", 1995.
Tanaka, et al., "Induction of Antitumor Cytotoxic T Lymphocytes With A MAGE-3 Encoded Synthetic Peptide Presented by Human Leukocytesntigen-24," Canc.Res. 57:4465-4468 (1997).
Traversari et al., "A Nonapeptide Encoded by Human Gene MAGE-1 Is Recognized on HLA-A1 by Cytolytic T Lymphocytes Directed Against Tumor Antigen MZ2-E", J. Exp. Med 176: 1453-1457 (11-92).
Celis et al., "Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes", Proc. Natl. Acad. Sci. USA 91: 2105-2109 (Mar. 1994.

Coulie et al., "A New Gene Coding For A Differential Antigen Recognized By Autologous Cytolytic T Lymphocytes on HLA-A2 Melanomas", J. Exp. Med. 180: 35-42 (Jul., 1994) (Not Prior Art).
Engelhard et al., "Structure of Peptides Associated With Class I and Class II MHC Molecules", Ann. Rev. Immunol. 12: 181-207 (1994).
Ruppert et al., "Prominent Role of Secondary Anchor Residues in Peptide Binding to HLA-A2.1 Molecules", Cell 74: 929-937 (Sep. 10, 1993).
Houbiers et al., "In Vitro Induction of human cytotoxic T lymphocyte responses against peptides of mutant and wild type p53", Eur. J. Immunol. 23: 2072-2077 (1993).
Townsend et al., "The Epitopes of Influenza Nucleoprotein Recognized by Cytotoxic T Lymphocytes Can be Defined With Short Synthetic Peptides", Cell 44: 959-968 (Mar. 28, 1986).
Bjorkman et al., "The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens", Nature 329: 512-518 (Oct. 8, 1987).
Van der Bruggen et al., "A Gene Encoding an Antigenic Recognized by Cytolytic T Lymphocytes on a Human Melanoma", Science 254: 1643-1647 (1991).

ART-UNIT: 164
PRIMARY-EXAMINER: Chan; Christina Y.
ASSISTANT-EXAMINER: Lubet; Martha
ATTY-AGENT-FIRM: Fulbright & Jaworski, LLP

ABSTRACT:

Tumor rejection antigens derived from tumor rejection precursor MAGE-3 have been identified. These "TRAS" bind to the MHC-class I molecule HLA-A2, and the resulting complexes stimulate the production of cytolytic T cell clones which lyse the presenting

cells. The peptides and complexes may be used diagnostically, therapeutically, and as immunogens for the production of antibodies, or as targets for the generation of cytolytic T cell clones.

8 Claims, 0 Drawing figures

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L11: Entry 1 of 6 File: USPT Feb 1, 2000

DOCUMENT-IDENTIFIER: US 6019987 A
TITLE: Isolated, MAGE-3 derived peptides which complex with HLA-A2 molecules and uses thereof

DEPR:
Cytolytic T cell clones thus derived are useful in therapeutic milieux such as adoptive transfer. See Greenberg, J. Immunol. 136(5): 1917 (1986); Reddel et al., Science 257: 238 (1992); Lynch et al., Eur. J. Immunol. 21: 1403 (1991); Kast et al., Cell 59: 603 (1989), all of which are incorporated by reference herein. In this methodology, the peptides set forth supra are combined with antigen presenting cells ("APCs"), to form stable complexes. Many such methodologies are known, for example, those disclosed in Leuscher et al., Nature 351: 72-74 (1991); Romero et al., J. Exp. Med. 174: 603-612 (1991); Leuscher et al., J. Immunol. 148: 1003-1011 (1992); Romero et al., J. Immunol. 150: 3825-3831 (1993); Romero et al., J. Exp. Med. 177: 1247-1256 (1993), and Romero et al., U.S. patent application Ser. No. 133,407, filed Oct. 5, 1993 and incorporated by reference herein. Following this, the presenting cells are contacted to a source of cytolytic T cells to generate cytolytic T cell clones specific for the complex of interest. Preferably, this is done via the use of an autologous T cell clone, found in, for example, a blood sample, taken from the patient to be treated with the CTLs. Once the CTLs are generated, these are reperfused into the subject to be treated in an amount sufficient to ameliorate the cancerous condition, such as by lysing cancer cells, inhibiting their proliferation, etc.

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L17: Entry 3 of 22 File: USPT Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6130087 A
TITLE: Methods for generating cytotoxic T cells in vitro

ABPL:
The present invention provides methods for generating antigen-reactive cytotoxic T cells in vitro comprising culturing immune cells and antigenic cells that have at least one MHC allele in common (and preferably, are syngeneic), in which the antigenic cells have been treated according to the methods of the invention. The antigenic cells are treated by subjecting them to osmotic shock followed by irradiation. As a result, a subset of T cells are activated and mature into antigen-reactive cytotoxic T cells. The effectiveness of the procedure may be enhanced by repeated restimulations and/or the addition of heat shock protein-peptide complexes. Methods and compositions are also disclosed for the treatment and prevention in a subject of cancer or infectious disease comprising administering to the subject matched cytotoxic T cells that are generated in vitro by the present methods.

BSPR:
The present invention relates to methods for generating antigen-reactive cytotoxic T cells for immunotherapy. The methods involve in vitro culture of immune cells with treated cancer cells or pathogen-infected cells, which results in the activation of a subset of T cells that are capable of effecting an antigen-specific cytotoxic T cell response.

BSPR:
Passive immunization is useful for a host who cannot produce antibodies, or for those who might develop disease before active immunization could stimulate antibody production. However, antibodies produced following some infections, particularly those due to mycobacteria, fungi, and many viruses, are not effective in protecting against the infection. Rather, the action of lymphocytes and macrophages largely determines recovery from these diseases.

BSPR:
Cells of the immune system arise from pluripotent stem cells through two main lines of differentiation: a) the lymphoid lineage producing lymphocytes (T cells, B cells, natural killer cells), and b) the myeloid lineage (monocytes, macrophages and neutrophils) and other accessory cells (dendritic cells, platelets and mast cells). In the circulatory system and secondary lymphoid organs of an adult animal, lymphocytes

recirculate and search for invading foreign substances. Pathogens and antigens tend to be trapped in secondary lymphoid organs, such as the spleen and the lymph nodes, where antigens are taken up by antigen-presenting cells (APC). Cell-mediated immune reactions require initial interaction between T cells and APC which trigger several effector pathways, including activation of cytotoxic T cells, and stimulation of T cell production of cytokines.

BSPR:
Cytotoxic T cells are antigen-specific effector cells that are important in resisting pathogens, cancer and allograft rejection. Most cytotoxic T cells are CD8.sup.+ cells that recognize antigen presented by MHC class I molecules which are expressed by almost all cell types. Cytotoxic T cells develop and undergo selection in the thymus, and then mature into functional cytotoxic T cells in the tissues after receiving two signals. The first signal is triggered by specific antigen-MHC complexes on the surface of antigen-presenting cells (APC). The second signal is provided by cytokines produced by CD4.sup.+ helper T cells, such as interferon-.gamma., and interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7) and interleukin-12 (IL-12).

BSPR:
Studies with experimental animal tumors as well as spontaneous human tumors have demonstrated that many tumors do express antigens that can induce an immune response. Some antigens are unique to the tumor, and some are found on both tumor and normal cells. Several factors can greatly influence the immunogenicity of the tumor induced, including, for example, the specific type of carcinogen involved, and immunocompetence of the host and latency period (Old et al., 1962, Ann. N.Y. Acad. Sci. 101:80-106; Bartlett, 1972, J Natl Cancer Inst 49:493-504). It has been demonstrated that T cell-mediated immunity is of critical importance for rejection of virally and chemically induced tumors (Klein et al., 1960, Cancer Res. 20:1561-1572; Tevethia et al., 1974, J. Immunol. 13:1417-1423). The cytotoxic T cell response is the most important host response for the control of growth of antigenic tumor cells (Anichimi et al., 1987, Immunol. Today 8:385-389).

BSPR:
Adoptive immunotherapy of cancer takes the therapeutic approach, wherein immune cells with an antitumor reactivity are administered to a tumor-bearing host, with the objective that the cells cause either directly or indirectly, the regression of an established tumor. Immunization of hosts bearing established tumors with tumor cells or tumor antigens has generally been ineffective since the tumor is likely to have elicited an immunosuppressive response (Greenberg, P. D., 1987, Chapter 14, in Basic and Clinical Immunology, 6th ed., ed. by Stites, Stobo and Wells, Appleton and Lange, pp. 186-196). Thus, prior to immunotherapy, it has been necessary to reduce the tumor mass and deplete all the T cells in the tumor-bearing host (Greenberg et al., 1983, page 301-335, in "Basic and Clinical Tumor Immunology", ed. Herbermann RR, Martinus

Nijhoff).

BSPR:
Animal models have been developed in which hosts bearing advanced tumors can be treated by the transfer of tumor-specific specific syngeneic T cells (Mule et al., 1984, Science 225:1487-1489). Investigators at the National Cancer Institute (NCI) have used autologous reinfusion of peripheral blood lymphocytes or tumor-infiltrating lymphocytes (TIL), T cell cultures from biopsies of subcutaneous lymph nodules, to treat several human cancers (Rosenberg, S.A., U.S. Pat. No. 4,690,914, issued Sep. 1, 1987; Rosenberg et al., 1988, N. Engl. J. Med., 319:1676-1680). For example, TIL expanded in vitro in the presence of IL-2 have been adoptively transferred to cancer patients, resulting in tumor regression in select patients with metastatic melanoma. Melanoma TIL grown in IL-2 have been identified as activated T lymphocytes CD.sup.+ HLA-DR.sup.+, which are predominantly CD8.sup.+ cells with unique in vitro antitumor properties. Many long-term melanoma TIL cultures lyse autologous tumors in a specific MHC class I- and T cell antigen receptor-dependent manner (Topalian et al., 1989, J: Immunol. 142:3714).

BSPR:
Application of these methods for treatment of human cancers would entail isolating a specific set of tumor-cancers reactive lymphocytes present in a patient, expanding these cells to large numbers in vitro, and then putting these cells back into the host by multiple infusions. Since T cells expanded in the presence of IL-2 are dependent upon IL-2 for survival, infusion of IL-2 after cell transfer prolongs the survival and augments the therapeutic efficacy of cultured T cells (Rosenberg et al., 1987, N. Engl. J. Med. 316:889-897). However, the toxicity of the high-dose IL-2 and activated lymphocyte treatment has been considerable, including high fevers, hypotension, damage to the endothelial wall due to capillary leak syndrome, and various adverse cardiac events such as arrhythmias and myocardial infarction (Rosenberg et al., 1988, N. Engl. J. Med. 319:1676-1680). Furthermore, the demanding technical expertise required to generate TILs, the quantity of material needed, and the severe adverse side effects limit the use of these techniques to specialized treatment centers.

BSPR:
The present invention relates to methods for generating antigen-reactive cytotoxic T cells that can be used for the prevention or treatment of a disease or disorder, such as infectious disease and cancer. The methods of the invention provide cytotoxic T cells that are capable of specifically killing antigenic cells, such as cancer cells or infected cells, with which the T cell has been primed.

BSPR:
The methods may further comprise restimulation of the primed T cells, such that a stable antigen-reactive T cell culture or T cell line can be maintained for extended periods in vitro. Therefore, T cells reactive to cancer cells and cells infected

with a pathogen can be generated rapidly in large numbers in vitro for various therapeutic and prophylactic applications for cancer and diseases and disorders associated with infection by the pathogen, respectively. The antigen-reactive T cell culture or T cell line can be stored, and used to resupply cytotoxic T cells for long term use in vivo.

BSPR:
The present invention also encompasses methods of treatment or prevention in a subject of cancer or infectious disease comprising administering to the subject antigen-reactive cytotoxic T cells provided by the methods of the invention. In a preferred aspect, the methods of immunotherapy of the invention are autologous in that the immune cells (and optionally, also the antigenic cells) are isolated from the same subject (preferably, human) who receives the antigen-reactive cytotoxic T cells.

BSPR:
Cytotoxic T cells that are reactive to infected cells can be used to treat a variety of infectious diseases (those diseases associated with or caused by infection with the same infectious agent as that infecting the infected cells), including but not limited to acute viral infections and infections by opportunistic infectious pathogens in immunosuppressed or immunodeficient subjects. Cytotoxic T cells reactive to cancer cells may be used alone or in conjunction with administration of cytokines, surgery and/or chemotherapy in treatment aimed at achieving cancer regression and eradicating metastases.

BSPC:
2.3 ADOPTIVE IMMUNOTHERAPY OF CANCER

BSPW:
2.3 ADOPTIVE IMMUNOTHERAPY OF CANCER

BSPW:
5.8 TARGET CANCERS

DRPR:
FIGS. 1A-1B. Reactivity of immune cells activated in vitro by SVB6 cancer cells. Naive spleens cells from C57BL/6 mice were stimulated in vitro with mock-loaded SVB6 cells (FIG. 1A) and untreated SVB6 cells (FIG. 1B). The results were determined by a 4-hour cytotoxicity assay, and expressed in percentages of .sup.51 Cr released from target cells (due to their lysis), at various ratios of activated immune cells (effector cells (E) to target cells (T), i.e., E:T ratio). SVB6 cells (open squares) were used as target cells, and EL4 cells (open circles) were used as negative control target cells.

DRPR:
FIGS. 2A-2B. Reactivity of immune cells activated in vitro by PS-C3H cancer cells. Naive spleen cells from C3H-HeJ mice were primed with mock-loaded PS-C3H cells (FIG. 2A) and untreated PS-C3H cells (FIG. 2B). The results were determined and expressed as described above for FIGS. 1A-1B. PS-C3H cells

(open squares) were used as target cells, and SVB6 (open diamonds) and UV 6138 (open circles) cancer cells were used as negative control target cells.

DRPR:
FIGS. 3A-3B. Reactivity of immune cells activated in vitro by EG7 cancer cells. Naive spleen cells from C57BL/6 mice were primed in vitro with mock-loaded EG7 cells (FIG. 3A) and untreated EG7 cells (FIG. 3B). The results were determined and expressed as described above for FIGS. 1A-1B. EG7 cells (open squares) were used as target cells, and SVB6 (open circles) and EL4 (open diamond) cancer cells were used as negative control target cells.

DRPR:
FIGS. 4A-4B. Reactivity of immune cells activated in vitro by N1 cancer cells. Naive spleen cells from C57BL/6 mice were primed in vitro with mock-loaded N1 cells (FIG. 4A) and untreated N1 cells (FIG. 4B). The results were determined and expressed as described above for FIGS. 1A-1B. N1 cells (open squares) were used as target cells, and EL4 (open circles) cancer cells were used as control target cells.

DEPR:
The present invention relates to methods for generating antigen-reactive cytotoxic T cells that can be used in the prevention or treatment of a disease or disorder, such as infectious disease and cancer. The inventors of the present invention discovered a method for priming T cells in vitro with antigenic cells such as cancer cells or infected cells, that leads to activation, proliferation and maturation of a specific set of antigen-reactive cytotoxic T cells. The cytotoxic T cells generated by the methods of the invention are capable of specifically killing or causing lysis of the cancer cells or infected cells, as the case may be, or any cells bearing the same antigens and similar MHC molecules with which the T cells are primed. The antigen-reactive T cells can be administered in vivo autologously (i.e., to the same individual from which the T cells (or parental cells to the T cells) were originally obtained) or sygeneically (i.e., to an identical twin of the individual from which the cancer or infected cells were initially obtained); or allogeneically to an individual who shares at least one common MHC allele with the individual from which the antigenic cells and T cells were originally obtained.

DEPR:
As used herein, the term "antigenic cells" refers to any cells, preferably cancer cells or infected cells, which can elicit an immune response in a subject. The sources of antigenic cells, and methods of preparation of antigenic cells for use in the present methods are discussed in section 5.1.

DEPR:
The "immune cells" that are contacted with the antigenic cells, and from which the cytotoxic T cells are generated, are a mixture of cells comprising T cells and antigen presenting

cells. The antigenic presenting cells may be but are not limited to macrophages, dendritic cells, and/or B cells. The term "priming" as used herein is synonymous with the process of antigen-induced activation of T cells.

DEPR:
cytotoxic T cells of the invention can specifically and directly kill target cells in vivo that bear the same antigen as the antigenic cells, thereby inhibiting cancer growth, or preventing or limiting the spread of the pathogen in the recipient.

DEPR:
In a preferred embodiment of the invention, the antigenic cells, the T cells, and the recipient of the cytotoxic T cells have the same MHC haplotype, i.e., the invention is directed to the use of autologous T cells stimulated in vitro with autologously-derived antigenic cells for the treatment or prevention of cancer or infectious disease in the same subject from which the T cells (or preferably, all the immune cells) and antigenic cells were originally derived. In a more preferred aspect, the immune cells and antigenic cells are isolated from a human subject in need of cellular immunotherapy.

DEPR:
Without limitation of the present invention to any particular scientific model or mechanism, the results described herein suggest that osmotically-shocked antigenic cells undergo fusion with macrophages (or other antigen presenting cells) present in the pool of immune cells, and that such fused cells are uniquely enabled to induce antigen-specific activation of T cells in vitro.

DEPR:
According to a specific embodiment of the invention, antigen-reactive cytotoxic T cells can also be generated and used prophylactically to prevent infection, or development or remission of cancer. In another embodiment, such T cells can be used therapeutically to treat infection or its sequelae or to treat cancer. Preferably, the antigenic cells used to generate the T cells are syngeneic to the subject to which they are to be administered, e.g., are obtained from the subject. However, if cancer cells or pathogen-infected cells that are syngeneic to the subject are not available for use, the methods of the invention provide that such antigenic cells having the same MHC haplotype as the intended recipient of the cells can be prepared in vitro using noncancerous or uninfected cells (e.g., normal cells) collected from the recipient. For example, depending on the mode of transmission of the pathogen, normal cells obtained from the recipient can be infected in vitro by incubation with the pathogen or other pathogen-infected cells, and then used to prime the immune cells. In another embodiment, normal cells can be induced to become cancer cells, e.g., by treatment with carcinogens, such as chemicals and/or radiation or infection with a transforming virus, and then used to prime the immune cells. Furthermore, in another embodiment, if the

cloned gene of the antigen of interest is available, normal cells from the subject can be transformed or transfected with the gene, such that the antigen of interest is expressed recombinantly in the cells, and then such cells can be used in the priming reaction. In a less preferred aspect, antigenic cells for use can be prepared from cells that are not syngeneic but that have at least one MHC allele in common with the intended recipient.

DEPR:
In an immune response, the process of antigen-induced T cell activation occurs in vivo typically in secondary lymphoid tissues, such as the lymph nodes and the spleen. By following the present methods, any antigenic cells of interest can be used to prime T cells or previously activated T cells in vitro, even cancer cells or infected cells that are considered unsafe for use in active immunization. Thus, one advantage of the present methods is that antigen-specific cytotoxic T cells can be generated in vitro against cancer cells or infected cells without having to immunize a subject with the antigenic cells or molecules.

DEPR:
There are many advantages of immunotherapy as provided by the present invention. Tumor bulk is minimal following surgery, and immunotherapy is most effective in this situation. In a specific embodiment, the preventive and therapeutic methods of the invention are directed at enhancing the immunocompetence of a cancer patient either before surgery or after surgery, and enhancing cell-mediated tumor-specific immunity against cancer cells, with the objective being inhibition of proliferation of cancer cells, and total eradication of residual cancer cells in the body. In another preferred aspect, cytotoxic T cells reactive against human cancer cells can be used, alone or in conjunction with surgery, chemotherapy, radiation or other anti-cancer therapies, to eradicate metastases or micrometastases, or to purge bone marrow of cancer cells during bone marrow transplantation. For example, to eradicate or inhibit the growth of metastases or micrometastases, the cytotoxic T cells provided by the invention are administered in vivo, to the subject having or suspected of having the metastases or micrometastases. For example, to purge bone marrow of cancer cells during bone marrow transplantation, bone marrow from the donor is contacted in vitro with the cytotoxic T cells provided by the invention, so that the cytotoxic T cells lyse any residual cancer cells in the bone marrow, prior to administering the bone marrow to the subject for purposes of hematopoietic reconstitution. The bone marrow transplantation is preferably autologous.

DEPR:
Moreover, if cancer patients undergo surgery with anesthesia, and subsequent chemotherapy, the resulting immunosuppression experienced by the patient may be lessened by cellular immunotherapy in the preoperative period, thereby reducing the incidence of infectious complications. There is also the possibility that tumor cells are shed into circulation at

surgery, and thus, effective immunotherapy applied at this time can eliminate these cells in vivo. The invention thus provides a method of prophylaxis or treatment comprising administering to a cancer patient the cytotoxic T cells provided by the present invention, reactive against an antigen of the patient's cancer cells, prior to, during, and/or subsequent to surgery and/or chemotherapy undergone by the cancer patient.

DEPR:
In a preferred aspect involving acute viral infection of humans, cytotoxic T cells reactive against virus-infected cells of a human subject can be rapidly generated and reinfused back to the subject for controlling the viral infection. An effective specific cell-mediated response against viral-infected cells can be generated by the present methods in vitro in less time than when relying on the in vivo mechanisms of the subject's immune system.

DEPR:
In another preferred aspect, the invention provides cytotoxic T cells reactive against an opportunistic pathogen that infects immunosuppressed or immunodeficient subjects, such as but not limited to cytomegalovirus, Toxoplasma gondii, Herpes zoster, Herpes simplex, Pneumocystis carinii, Mycobacterium avium-intracellulare, Mycobacterium tuberculosis, Cryptosporidium, and Candida species. The cytotoxic T cells of the invention can be used therapeutically, and preferably autologously, in human patients suffering from acquired immunodeficiency syndrome (AIDS) and associated infections and cancers, or prophylactically in subjects that are infected with the human immunodeficiency virus (HIV), or HIV seropositive subjects or otherwise at high risk for developing AIDS.

DEPR:
The present invention provides methods for priming T cells in vitro with cancer cells or infected cells or other antigenic cells of interest. For treatment or prevention of cancer, the methods of the invention provide cytotoxic T cells primed against molecules (antigens) expressed by antigenic cells that will induce an immune response against the cancer cells or tumor, preferably human cancers, e.g., tumor-specific antigens and tumor associated antigens. For treatment or prevention of infectious diseases, the methods of the invention provide cytotoxic T cells primed against molecules (antigens) expressed by antigenic cells that will induce an immune response against host cells infected by the pathogen that causes the infectious disease or against the pathogen, including but not limited to, viruses, bacteria, fungi, protozoans, parasites, etc., and preferably pathogens that infect humans. Since whole cancer cells or infected cells or other antigenic cells are used in the present methods, it is not necessary to isolate or characterize or even know the identities of these antigens in advance of using the present methods.

DEPR:
The source of the antigenic cells may be selected, depending on the nature of the disease with which the antigen is associated,

and the intended use of the resulting cytotoxic T cells. In one embodiment of the invention, any tissues, or cells isolated from a cancer, including cancer that has metastasized to multiple sites, can be used in the present method. For example, leukemic cells circulating in blood, lymph or other body fluids can also be used, solid tumor tissue (e.g., primary tissue from a biopsy) can be used. Examples of cancers that are amenable to the methods of the invention are listed infra.

DEPR:
In another embodiment of the invention, any cell that is infected with a pathogen, in particular, an intracellular pathogen, such as a virus, bacterium, fungus, parasite, or protozoan, can be used. An exemplary list of infectious diseases that can be treated or prevented by cytotoxic T cells of the invention is provided below.

DEPR:
Cell lines derived from cancer tissues, cancer cells, or infected cells can also be used as antigenic cells, provided that the cells of the cell line have the same antigenic determinant(s) as the antigen of interest on the antigenic cells. Cancer or infected tissues, cells, or cell lines of human origin are preferred.

DEPR:
Cancer cells or infected cells can be identified and isolated by any method known in the art. For example, cancer cells or infected cells can be identified by morphology, enzyme assays, proliferation assays, or the presence of pathogens or cancer-causing viruses. If the characteristics of the antigen of interest are known, antigenic cells can also be identified or isolated by any biochemical or immunological methods known in the art. For example, cancer cells or infected cells can be isolated by surgery, endoscopy, other biopsy techniques, affinity chromatography, and fluorescence activated cell sorting (e.g., with fluorescently tagged antibody against an antigen express by the cells).

DEPR:
In another embodiment of the invention, antigen-reactive T cells are generated for prophylaxis against cancer or infectious disease. In this instance, the appropriate autologous antigenic cells may not exist, since the recipient of the cytotoxic T cells may not have the cancer or the infectious disease. Moreover, in an embodiment wherein the T cells are used for prophylaxis, desired quantities of autologous antigenic cells may not be obtainable from the recipient. In such instances, a source of antigenic cells having at least one common MHC allele, or preferably the same MHC haplotype as the recipient, which is used to prime the cytotoxic T cells, can be prepared in vitro from noncancerous or uninfected cells (e.g., normal cells), as appropriate, obtained from the recipient or other individual sharing at least one MHC allele with the recipient.

DEPR:

In order to prepare suitable antigenic cells that are cancer cells, noncancerous cells, preferably of the same cell type as the cancer desired to be inhibited can be isolated from the recipient or, less preferably, other individual who shares at least one MHC allele with the intended recipient, and treated with agents that cause the particular or a similar cancer or a transformed state; such agents may include but not limited to, radiation, chemical carcinogens, and viruses. Standard techniques can be used to treat the cells and propagate the cancer or transformed cells so produced.

DEPR:
In order to prepare pathogen-infected cells, uninfected cells of a cell type susceptible to infection by the pathogen can be infected in vitro with the pathogen that causes the disease. Depending on the mode of transmission and the biology of the pathogen, standard techniques can be used to facilitate infection by the pathogen, and propagation of the infected cells. For example, influenza viruses may be used to infect normal human fibroblasts; and mycobacteria may be used to infect normal human Schwann cells.

DEPR:
The immune cells used for priming according to the present invention, comprise a mixture of living cells of lymphoid and myeloid lineages, wherein the lymphoid cells comprise T cells that can be activated to differentiate into CD4.sup.+ helper T cells and CD8.sup.+ cytotoxic T cells, and wherein the myeloid cells comprise antigen-presenting cells (e.g., macrophages) that are functional in antigen-induced T cell activation. Preferably, the T cells have the same MHC haplotype as the antigenic cells and the target cells in the recipient.

DEPR:
T cells and antigen presenting cells, such as macrophages, can be obtained together, or separately (and then combined for use in the priming reaction), or optionally purified by any of various methods known in the art. In a preferred aspect human macrophages obtained from human blood cells are used. By way of example but not limitation, macrophages can be obtained as follows:

DEPR:
Any antigenic cell, e.g., cancer or infected cells described in section 5.1, may be used in the present methods. Typically, by way of example but not limitation, cancer cells can be isolated from a tumor that is surgically removed from a human patient who will be the recipient of the cytotoxic T cells. Prior to use, solid cancer tissue, pathogen-infected tissue or aggregated cancer cells should be dispersed, preferably mechanically, into a single cell suspension by standard techniques. Enzymes, such as but not limited to, collagenase and DNase may also be used to disperse cancer cells. Typically, approximately two to three million antigenic cells are used per priming reaction in the method. Thus, if necessary, the cancer or infected cells may be cultured by standard techniques under growth conditions in vitro to obtain the desired number of

cells prior to use. Primary tissue or cell lines can be used.

DEPR:
The present invention provides a method for generating cytotoxic T cells comprising the following steps in the order stated: subjecting live antigenic cells to osmotic shock in vitro; subjecting the antigenic cells to irradiation in vitro; culturing the antigenic cells with immune cells comprising T cells and antigen presenting cells in vitro, wherein the antigenic cells and T cells have at least one common MHC allele, such that cytotoxic T cells are generated.

DEPR:
The cancer or infected cells (e.g., 2.times.10.sup.6 cells) are incubated at 37.degree. C. for 5 to 60 minutes in a hyperosmotic buffer (0.5 ml per 2.times.10.sup.6 cells) containing 0.5 M sucrose, 10% w/v polyethylene glycol 1000, 10 mM HEPES in tissue culture medium, such as RPMI 1640. (Alternatively, any solutes can be used to prepare a hyperosmotic buffer that would exert a similar osmotic pressure, e.g., about three times physiologic osmotic pressure.) In order to reverse the osmotic effect rapidly, the medium containing the cells is diluted by adding 30 times the volume of a hypotonic buffer (15 ml per 2.times.10.sup.6 cells) consisting of 60% tissue culture medium in water. The cells are then incubated at 37.degree. C., e.g., for 1-10 minutes, before they are collected by centrifugation at 500-5000 rpm in a standard laboratory centrifuge. The antigenic cells are not ruptured at the end of this process. The osmotically-shocked antigenic cells are resuspended in culture medium (5 ml for 2.times.10.sup.6 cells) and allowed to recover for 0.5 to 24 hours at 37.degree. C., 5% CO.sub.2.

DEPR:
After the osmotic shock, the antigenic cells (e.g., one to two million) are irradiated with 1,000 to 10,000 rads in culture medium containing 5% fetal calf serum, and then washed in culture medium containing 10% fetal calf serum. For example, the antigenic cells are irradiated for 30 minutes using a Gammator 50 set to deliver 800 rads/min. Antigenic cells that are subjected to osmotic shock, followed by irradiation are referred to herein as mock-loaded antigenic cells. The in vitro priming reaction is carried out by culturing the mock-loaded cells with an immune cell composition comprising T cells and antigen presenting cells of a subject. As described in section 5.2, immune cells can be obtained from the spleen or preferably, peripheral blood. If lymphoid tissues or organs are used as a source of immune cells, it is preferable to disperse the cells so as to form a single cell suspension prior to use.

DEPR:
Approximately 1.times.10.sup.6 cancer or infected cells treated as described above (with osmotic shock and irradiation) are used to prime 1.times.10.sup.8 immune cells (e.g., whole blood from which the red blood cells and serum have been removed). The cells are mixed and co-cultured at 37.degree. C., 5% CO.sub.2, for preferably 3-9 days, preferably in multiwell

plates. The ratio of treated cells to immune cells in the mixed cell culture is preferably 1:100, and may be adjusted to optimize the ratio for generation of responding cytotoxic T cells, after measuring the resulting reactivity of the responding cytotoxic T cells at various ratios. Optionally, noncovalent complexes of heat shock protein and peptides isolated from cells that are the same as the antigenic cells, or noncovalent complexes of heat shock protein and exogenous antigenic peptides, are added to the mixed cell culture to enhance the efficiency of the in vitro priming reaction. Heat shock protein-peptide complexes can be isolated or prepared in vitro by methods described in the next section. A concentration range of 1-50 .mu.g heat shock protein peptide complex per ml culture medium is preferred. After several days in culture, e.g., on day six, a .sup.51 Cr release assay or any other suitable assay known in the art may be performed if desired, to test for cytotoxic T cell activity in the mixed cell culture.

DEPR:
Any methods known in the art can be employed to purify endogenous hsp-peptide complexes from antigenic cells, e.g., cancer cells or infected cells, for use in the present methods. For example, the purification of hsp70-peptide complexes has been described previously, see, for example, Udono et al., 1993, J. Exp. Med. 178:1391-1396. See also International Publication No. WO 95/24923 dated Sep. 21, 1995.

DEPR:
Another method that can be applied for the purification of heat shock protein-peptide complexes, such as hsp70-peptide complexes, comprises contacting cellular proteins with ADP or a nonhydrolyzable analog of ATP affixed to a solid substrate, such that hsp70 in the lysate can bind to the ADP or nonhydrolyzable ATP analog, and eluting the bound hsp70. A preferred method uses column chromatography with ADP affixed to a solid substratum (e.g., ADP-agarose). Thus, hsp70-peptide complexes can be readily obtained from cancer cells or cells infected by an infectious agent or other cells by a rapid, one-step ADP-agarose chromatography. For example, Meth A sarcoma cells (500 million cells) can be homogenized in hypotonic buffer and the lysate is centrifuged at 100,000 g for 90 minutes at 4.degree. C. The supernatant is applied to an ADP-agarose column. The columns are washed in buffer and are eluted with 3 mM.

DEPR:
By way of example but not limitation, the following four (4) hour .sup.51 Cr-release assay can be used (see, Palladino et al., 1987, Cancer Res. 47:5074-5079 and Blachere et al., 1993, J. Immunotherapy 14:352-356). In this assay, cells in the primed immune cell culture, i.e., the effector cells, are added to a target cell suspension to give various effector:target (E:T) ratios (usually from 1:1 to 40:1). The target cells are prelabelled by incubating 1.times.10.sup.6 target cells in culture medium containing 200 MCi .sup.51 Cr/ml for one hour at 37.degree. C. The labelled cells are washed three times following labeling. Each assay point (E:T ratio) is performed

in triplicate. The controls measure spontaneous .sup.51 Cr release wherein no lymphocytes are added to the assays, and 100% release wherein the labelled target cells are lysed with detergent, such as TNEN (10 mM Tris-HCl, 250 mM NaCl, 0.1 mM EDTA and 1% NP-40). After incubating the effector/target cell mixtures for 4 hours, the cells are collected by centrifugation at 200 g for 5 minutes. The amount of .sup.51 Cr released into the supernatant is measured by a gamma counter. The percent cytotoxicity is measured as cpm in the test sample minus spontaneously released cpm divided by the total detergent released cpm minus spontaneously released cpm. ##EQU1##

DEPR:
In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer or infection, or directly into the cancer or tumor.

DEPR:
Infectious diseases that can be treated or prevented by cytotoxic T cells of the present invention are caused by infectious agents including, but not limited to viruses, bacteria, fungi, protozoans and parasites.

DEPR:
Viral diseases that can be treated or prevented by the methods and compositions of the present invention include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I), and human immunodeficiency virus type II (HIV-II).

DEPR:
Cancers that can be treated or prevented by cytotoxic T cells and methods of the present invention include, but not limited to human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas,

cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute

DEPR:
In a specific embodiment, the cancer is metastatic. In another specific embodiment, the cancer is a tumor. In yet another embodiment, the recipient has undergone autologous bone marrow transplantation as a part of cancer therapy.

DEPR:
The following example demonstrates the methods of the present invention for generating cytotoxic T cells in vitro, using four different types of cancer cells in two different mouse strains.

DEPR:
The following cancer cells were used: SVB6, a C57BL/6 mouse cancer cell line transformed with SV40 viruses); EL4 (a C57BL/6 mouse thymoma cell line); EG7 and N1 (mouse thymoma cell lines derived EL4); PS-C3H (a SV40-transformed C3H-He mouse sarcoma cell line) and UV6138 (a C3H-He mouse cancer cell line).

DEPR:
Two to three million cancer cells were resuspended in 0.5 ml of the hyperosmotic buffer and incubated for ten minutes at 37.degree. C. To reverse the osmotic effect, the hypotonic buffer (15 ml) was added directly to the cells in the hyperosmotic buffer, and incubated for three more minutes at 37.degree. C. The cells were immediately spun down in a standard laboratory centrifuge at 700 g at 4.degree. C. The supernatant was poured off through a gauze pad (Johnson and Johnson Products Inc.) and the cells resuspended in 5 ml RPMI 1640 supplemented with 5% fetal calf serum (FCS), 1% glutamine, 1% penicillin-streptomycin, 1% nonessential amino acids, pyruvate and 0.1% 2-mercaptoethanol. The cells were allowed to recover for two hours at 37.degree. C. and 5% CO.sub.2.

DEPR:
One to two million of the treated cancer cells were irradiated (Gammator 50; 800 rads/min) for 30 minutes in 3 ml of complete RPMI 1640 containing 5% FCS. The cells were washed once and resuspended in complete RPMI 1640 containing 10% FCS.

DEPR:
The treated cancer cells were added to each well so that each well had a total volume of 2 ml. Approximately a total of 1.times.10.sup.6 treated cancer cells were used to prime a total of 1.times.10.sup.8 spleen cells. The plates were incubated at 37.degree. C. and 5% CO.sub.2 for six days. A .sup.5 Cr release assay was performed on day six to test for T

cell reactivity as follows: Target cells were labeled by incubation in .sup.51 Cr for 60 min at 37.degree. C., and washed three times in RPMI 1640 medium. In vitro primed immune cells were incubated with 2.times.10.sup.3 51Cr-labeled target cells in 0.2 ml of complete medium containing 10% FCS in V-bottom plates for 4 hours. A range of effector-to-target cell ratios were used in the experiments. An aliquot of the culture fluid was counted in a gamma scintillation counter. Cytotoxic T cell activity was calculated as described in section 5.5. The spontaneous .sup.51 Cr release was determined by incubation of targets in complete medium. The maximal .sup.51 Cr release was determined by incubation of targets in TNEN.

DEPR:
The following experiments were performed to illustrate the methods of the present invention. Spleen cells isolated from C57BL/6 mice were primed in vitro according to the procedure described in section 6.2 using the following mock-loaded cancer cells: SVB6, EG7 and N1. Similarly, spleen cells of C3H-He mice were primed in vitro with mock-loaded PS-C3H cancer cells. Mock-loading as used herein refers to the treatment of antigenic cells with osmotic shock and irradiation and was carried out as described in section 6.2. As controls, spleen cells from C57BL/6 and C3H-He were stimulated by co-culturing with cancer cells that were not treated with osmotic shock.

DEPR:
The antigen-specific cytotoxicity of the responding T cells in the in vitro primed cell cultures were tested by the four-hour .sup.51 Cr release assay as described in section 6.2. The amount of .sup.51 Cr release measures the degree of target cell lysis mediated by the cytotoxic T cells in the four-hour period. Antigenic cells that were used in the in vitro priming reaction were used as target cells. Other cancer cells were used as control target cells to demonstrate the specificity of the cytotoxic effect.

DEPR:
Using C57BL/6 spleen cells and mock-loaded SVB6, EG7 and N1 cells, the amount of .sup.51 Cr released as a result of target cell lysis mediated by cytotoxic T cells generated by the mock-loaded cancer cells (FIGS. 1A, 3A and 4A) was significantly and consistently higher than that mediated by T cells generated by untreated antigenic cells (FIGS. 1B, 3B and 4B). Similarly, cytotoxic T cells generated from C3H-He spleen cells using mock-loaded PS-C3H cells (FIG. 2A) were more efficient at lysing target cells than the control (FIGS. 2A-2B). Thus, the cytotoxic effect generated by mock-loaded antigenic cells of the present invention is significantly stronger than using untreated cancer cells, especially at higher effector:target cell ratios.

DEPR:
Furthermore, the cytotoxic effect generated by stimulation with mock-loaded cancer cells is antigen specific. Cytotoxic T cells generated by stimulation with mock-loaded SVB6 cells have little or no cytotoxic effect on EL4 cells (FIG. 1A). Likewise,

cytotoxic T cells generated using mock-loaded EG7 or N1 cells were significantly less reactive against, respectively, the control EL4 and SVB6 cells (FIG. 3A), and the control EL4 cells (See FIG. 4A).

DEPR:
In summary, antigenic cells treated according to the methods of the invention can stimulate in vitro the activation of T cells that can mediate an effective antigen-specific cytotoxic T cell response. Thus, administration of such in vitro generated antigen-reactive cytotoxic T cells to a MHC-matched recipient, as described herein, represents a therapeutic or preventative approach for a wide range of cancers or infectious diseases.

DEPC:
5.8 TARGET CANCERS

CLPR:
3. A method for generating cytotoxic T cells specific to antigenic cells displaying an antigenicity of interest comprising culturing in vitro immune cells comprising T cells and antigen presenting cells with antigenic cells displaying an antigenicity of interest, such that the T cells become activated, thereby generating cytotoxic T cells that are specific to the antigenicity of interest displayed by the antigenic cells, wherein the antigenic cells are animal cells that are the product of a method comprising subjecting in vitro the antigenic cells displaying the antigenicity of interest to osmotic shock followed by irradiation, and wherein the antigenic cells and T cells have at least one common MHC allele.

CLPR:
9. The method of claim 1 wherein the antigenic cells are cancer cells.

CLPR:
10. The method of claim 2 wherein the antigenic cells are cancer cells.

CLPR:
11. The method of claim 3 wherein the antigenic cells are cancer cells.

CLPR:
12. The method of claim 9 wherein the cancer cells are of a cancer selected from the group consisting of sarcoma, carcinoma, leukemia, lymphoma and myeloma.

CLPR:
13. The method of claim 9 wherein the cancer cells are of a prostate cancer.

CLPR:
14. The method of claim 9 wherein the cancer cells are of a breast cancer.

CLPR:
15. The method of claim 9 wherein the cancer cells are of a colon cancer.

CLPR:
19. The method of claim 16, 17 or 18 wherein the antigenic cells are infected by a virus, bacterium, fungus, parasite, or protozoan.

CLPR:
22. The method of claim 1 wherein the antigenic cells recombinantly express an antigen of a cancer cell.

CLPR:
23. The method of claim 1 wherein the antigenic cells have been induced in vitro to become cancer or transformed cells.

CLPR:
34. The method of claim 31 or 32 wherein the antigenic cells are cancer cells.

CLPV:
(c) culturing the antigenic cells with immune cells comprising T cells and antigen presenting cells in vitro, wherein the antigenic cells and T cells have at least one common MHC allele,

CLPV:
(d) culturing the antigenic cells in vitro with immune cells comprising T cells and antigen presenting cells at a ratio of about 1:100 antigenic cells:immune cells for 3 to 9 days at 37.degree. C.,

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